



Pharmacological Study

A comparative study on chronic administration of *Go Ghrita* (cow ghee) and *Avika Ghrita* (ewe ghee) in albino rats

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Abstract

Ghrita (ghee) is the foremost substance of Indian cuisine from centuries. Ayurvedic classics described eight kinds of ghee from eight different animal milk, among them ghee made from cow milk is said to be the superior and ghee of ewe milk is said to be the inferior and also detrimental to heart. The present study was undertaken to evaluate chronic administration of cow ghee (*Go Ghrita*) and ghee of ewe milk (*Avika Ghrita*) to experimental animals. Experiment was carried out on Wistar strain albino rats and study was done at two dose levels. The test drugs were administered orally for 45 consecutive days. Parameters, such as gross behavior, body weight, weight of important organs, total fecal fat content, electrocardiogram, serum biochemical parameters, and histopathology of different organs were studied. Both the test drugs did not alter the gross behavior, body weight, weight of organs, and cytoarchitecture of different organs to significant extent. *Avika Ghrita* at a low dose significantly decreased triglyceride content, significantly prolonged QTc and at both dose levels it significantly shortened the PR interval. This study shows chronic administration of *Avika Ghrita* and *Go Ghrita* has no marked differences between them except the QTc prolongation in *Avika Ghrita*. This may be the basis for the classics to categorize *Avika Ghrita* as *Ahridya*.

Key words: *Ahridya*, albino rats, *Avika Ghrita*, cholesterol, ghee, *Go Ghrita*

Introduction

Ahara (diet), *Nidra* (sleep), and *Brahmacharya* (chastity or self-restriction in the use of sense organs) have been considered as three subpillars of life. Food is at leading place among these three because of its prime importance in life-sustaining substances. *Sukha* (health) or *Dukha* (disease) depends on *Hita* (good) or *Ahita* (awful) effects of food taken by the person. According to *Maharshi Kashyapa* there is no medicine like *Ahara* (food) on the earth.^[1] This is a clear indication of medicinal use of diet articles. In spite of all these good qualities and importance, the same *Ahara* (food) can cause various kinds of serious disorders in the body. This is why *Maharshi Charaka* said that the body as well as the disease is the product of *Ahara* (food).^[2] *Ghrita* (ghee) is the foremost

substance of Indian cuisine from centuries. Any region might be spotted out where *Ghrita* is not used as daily routine diet article. Regardless of warnings from modern medical science, Indian population uses *Ghrita* in their regular diet. *Ghrita* can be made from the milk of different animals. Ayurvedic classical texts described eight kinds of ghee from eight different animal milk and ghee made from cow milk is said to be superior among them,^[3] whereas ghee of ewe milk is said to be inferior.^[4] *Maharsi Caraka* has mentioned it as *Ahridya* (detrimental to heart).^[5] In the present era, due to less production and high-priced, adulteration of *Ghrita* is very common. To counteract this problem, dairy industry is expanding its area for products made from the milk other than cow and buffalo for satisfying the demand of ghee. Milk of ewe is one of them which is planned to use for production of ghee. When there is clear reference available regarding *Ahridya* (detrimental to heart) effect of *Avika Ghrita* (ghee of ewe milk) in almost all the classical texts, it is the need of the hour to evaluate it scientifically. Till date no pharmacological data is available regarding this aspect. Hence the present study was undertaken to evaluate chronic administration of

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Go Ghrita and *Avika Ghrita* to experimental animals and their effect on various ponderal, biochemical, histopathological, and electro-physiological parameters.

Materials and Methods

Animals

Thirty Wistar strain albino rats of either sex weighing between 160 ± 30 g were selected from animal house attached to the pharmacology laboratory. Animals were maintained on *Amrut* brand pellets obtained from *Pranava Agro Industries*, Pune, India, and exposed to ambient temperature, humidity and natural day and night cycles. The experiment was carried out between 8:00 am to 12:00 noon hours of the day. The experimental protocol was submitted to the Institutional Animal Ethics Committee, and approval was obtained for conducting the experiment (Approval number – IAEC/3/07-10/MD 04).

Test drugs

The test drugs *Go Ghrita* and *Avika Ghrita* were prepared by adopting standard ghee preparation methods.^[6]

Dose fixation

The human dose of *Ghrita* as practiced traditionally is approximately 10 g per person. Accordingly the dose was calculated by extrapolating the human dose to animals (900 mg/kg) based on the body surface area ratio by referring to the standard table of Paget and Barnes.^[7] The study was carried out at two dose levels, that is, therapeutically equivalent dose (900 mg/kg) and maximum dose, that is, population taking *Ghrita* as a daily diet article in a large quantity (4140 mg/kg).

Experimental design

The selected animals were kept on acclimatization for 7 days and thereafter they were grouped randomly into 5 groups of 6 animals in each group. The first group served as normal control and second and third groups were administered with *Avika Ghrita* at 900 mg/kg and 4140 mg/kg doses, respectively. The fourth and fifth groups were administered with *Go Ghrita* at 900 mg/kg and 4140 mg/kg doses, respectively. Test drugs were administered orally once a day for 45 consecutive days.

The effect on gross behavior was observed once in a week by exposing individually on open arena. The total fecal fat content was estimated at 0, 15, 30, and 45th day. Electrocardiogram (ECG) recording was done on the 43rd day of drug administration after anesthetizing the animals with diethyl ether. ECG was recorded by using a portable ECG machine (Cardiofax, Medicaid Systems, Chandigarh, India). Only the four standard (limb) leads were attached to four extremities of the animals, and the chest leads were not used. The speed of the ECG machine was set to 50 mm/s.

On the 46th day the blood was obtained from the retro-orbital puncture under light ether anesthesia using capillary tubes. The body weight was noted and they were sacrificed by excess dose of ether. Blood samples were collected for assessing different types of biochemical parameters, such as blood urea level,^[8] serum creatinine,^[9] serum total cholesterol,^[10] serum High Density Lipoprotein (HDL) cholesterol,^[11] serum triglyceride,^[12] serum total protein,^[13] serum albumin,^[14] serum

globulin, serum alkaline phosphatase,^[15] Serum Glutamic Oxaloacetic Transaminase (SGOT),^[16] Serum Glutamic Pyruvic Transaminase (SGPT),^[17] serum bilirubin (Total and Direct).^[18] The important organs, such as heart, liver, aorta, kidney, testis, uterus, and ovary were dissected out, weighed (except aorta) and all organs were fixed in 10% formalin solution. The histopathological slides were prepared by referring the standard procedure.^[19] The slides were viewed under binocular research Carl-Zeiss's microscope (Germany) to note down the changes in the microscopic features of the tissues.

Statistical analysis

The obtained data have been presented as Mean \pm SEM (Standard Error of Mean), difference between the groups was statistically determined by unpaired Student's *t* test and analysis of variance (ANOVA) followed by Dunnett's *t* test for all the treated groups with the level of significance set at $P < 0.05$. The level of significance was noted and interpreted accordingly.

Results

The gross behavior in rats shows mild hyperactivity in higher doses of *Avika Ghrita* administered group in comparison to initial values as well as control group. In both the doses of *Go Ghrita* administered group also mild hyperactivity was observed in comparison with the respective initial values and control group.

In the control group normal progressive gain in body weight was observed. Similarly in both *Go Ghrita* and *Avika Ghrita* at both dose-treated groups gain in body weight was observed; however, the observed increase in body weight was found to be statistically insignificant [Table 1]. Regarding the organ weight, out of five organs studied none of the organ weight was significantly affected by the administration of test drugs for 45 days [Table 2].

The effect of test drugs on the total fecal fat content is provided in Table 3. Both the test drugs at both dose levels markedly increased the total fecal fat content. However only the observed increase of total fecal fat content of Low Dose of *Avika Ghrita* (AGLD) and High Dose of *Go Ghrita* (GGHD) treated groups were found to be statistically significant in comparison with the control group.

In total 13 biochemical parameters, none of the parameters was affected to a significant extent except triglyceride content of AGLD treated group, which was significantly decreased in comparison with the control group [Table 4].

Among the six electro-physiological parameters recorded, *Avika Ghrita* at both dose levels significantly shortened the PR interval and significantly prolonged QTc at the low-dose level [Table 5, Figures 1, 2]. Treatment with *Go Ghrita* at both the dose levels did not affect any of the parameters to a significant extent [Figures 3, 4].

Examination of sections of all the seven organs from the control group exhibited normal cytoarchitecture. Examination of sections from *Avika Ghrita* and *Go Ghrita* at both dose level treated groups also showed almost normal cytoarchitecture except for mild fatty changes in one or two sections of the liver from these treated groups.

Table 1: Effect on body weight

| Body weight (g) | Control | AGLD | AGHD | GGLD | GGHD |
|----------------------|--------------|--------------|--------------|--------------|--------------|
| Initial | 176.67±06.19 | 171.33±11.86 | 153.67±05.62 | 169.00±09.25 | 165.00±13.60 |
| 14 th day | 195.33±10.52 | 183.00±12.49 | 179.33±07.13 | 187.67±11.55 | 198.00±15.72 |
| 28 th day | 215.33±15.14 | 207.33±11.04 | 200.00±8.25 | 203.67±14.00 | 211.67±18.79 |
| 46 th day | 213.33±12.56 | 185.33±13.97 | 188.33±14.33 | 185.33±15.76 | 204.33±21.23 |

Data expressed as mean ± standard error of mean;AGLD: Low Dose of *Avika Ghrita*;AGHD: High Dose of *Avika Ghrita*; GGLD: Low Dose of *Go Ghrita*; GGHD: High Dose of *Go Ghrita*

Table 2: Effect on organ weight

| Organ weight (mg/100 g) | Control | AGLD | AGHD | GGLD | GGHD |
|-------------------------|----------------|----------------|----------------|----------------|----------------|
| Liver | 2563.55±123.15 | 2705.22±158.17 | 2724.15±152.98 | 2557.58±088.16 | 2837.17±109.63 |
| Heart | 307.22±09.26 | 308.58±08.29 | 291.44±04.10 | 315.30±12.34 | 303.95±10.74 |
| Kidney | 759.37±109.64 | 740.50±17.49 | 674.33±22.94 | 714.20±27.00 | 687.18±15.70 |
| Testis | 959.83±151.66 | 1046.39±115.6 | 944.86±150.84 | 1106.7±115.28 | 1006.24±115.19 |
| Uterus | 241.09±101.51 | 171.86±19.30 | 181.28±53.64 | 119.2±20.21 | 119.94±27.37 |

Data expressed as mean ± standard error of mean;AGLD: Low Dose of *Avika Ghrita*;AGHD: High Dose of *Avika Ghrita*; GGLD: Low Dose of *Go Ghrita*; GGHD: High Dose of *Go Ghrita*

Table 3: Effect on total fecal fat content

| Parameters (mg/g) | Control | AGLD | AGHD | GGLD | GGHD |
|----------------------|--------------|--------------|-------------|-------------|--------------|
| 0 day | 0.035±0.006 | 0.033±0.004 | 0.038±0.004 | 0.029±0.006 | 0.033±0.006 |
| 15 th day | 0.0167±0.002 | 0.023±0.003 | 0.027±0.005 | 0.027±0.004 | 0.018±0.007 |
| 30 th day | 0.0150±0.003 | 0.022±0.003 | 0.030±0.004 | 0.017±0.004 | 0.018±0.004 |
| 45 th day | 0.032±0.004 | 0.052±0.008* | 0.058±0.014 | 0.055±0.019 | 0.058±0.011* |

Data expressed as mean±standard error of mean.AGLD: Low Dose of *Avika Ghrita*;AGHD: High Dose of *Avika Ghrita*; GGLD: Low Dose of *Go Ghrita*; GGHD: High Dose of *Go Ghrita*. *P<0.05 (compared with control group)

Table 4: Effect on serum biochemical parameters

| Parameters | Control | AGLD | AGHD | GGLD | GGHD |
|-----------------------------|--------------|--------------|--------------|--------------|--------------|
| Total protein (g/dL) | 7.25±0.15 | 7.23±0.26 | 6.80±0.17 | 7.07±0.27 | 7.15±0.20 |
| Albumin (g/dL) | 3.73±0.12 | 3.50±0.14 | 3.62±0.15 | 3.52±0.11 | 3.98±0.08 |
| Globulin (g/dL) | 3.52±0.17 | 3.90±0.39 | 3.18±0.10 | 3.55±0.27 | 3.17±0.20 |
| SGOT (IU/L) | 191.17±16.60 | 174.17±27.13 | 238.33±27.46 | 183.67±34.17 | 159.17±23.61 |
| SGPT (IU/L) | 43.00±03.28 | 45.50±08.95 | 55.83±05.55 | 52.33±10.06 | 43.00±04.35 |
| ALP (IU/L) | 140.33±29.72 | 161.50±29.49 | 201.67±19.82 | 193.00±23.4 | 223.50±32.30 |
| Bilirubin total (mg/dL) | 0.45±0.04 | 0.47±0.05 | 0.53±0.10 | 0.52±0.08 | 0.48±0.08 |
| Serum bilirubin (D) (mg/dL) | 0.15±0.02 | 0.13±0.02 | 0.15±0.04 | 0.15±0.02 | 0.15±0.02 |
| Blood urea (mg/dL) | 54.50±4.49 | 65.17±5.83 | 59.00±1.93 | 72.00±7.12 | 60.67±6.34 |
| Creatinine (mg/dL) | 0.68±0.04 | 0.78±0.05 | 0.70±0.05 | 0.78±0.10 | 0.63±0.02 |
| Total cholesterol (mg/dL) | 56.33±4.06 | 53.33±3.46 | 45.67±4.08 | 47.67±3.37 | 53.83±3.07 |
| Triglyceride (mg/dL) | 69.33±14.49 | 36.00±1.86* | 49.50±10.72 | 46.33±3.97 | 44.33±9.61 |
| HDL (mg/dL) | 35.67±4.24 | 30.00±3.03 | 30.33±2.87 | 28.83±2.50 | 32.67±1.38 |

Data expressed as mean±standard error of mean.;AGLD: Low Dose of *Avika Ghrita*;AGHD: High Dose of *Avika Ghrita*; GGLD: Low Dose of *Go Ghrita*; GGHD: High Dose of *Go Ghrita*; SGOT: Serum Glutemic Oxalic Acid; SGPT: Serum Glutemic Pyruvic Acid;ALP:Alkaline Phosphatase; HDL: High Density Lipoprotein; *P<0.05 (Compared with control group)

Discussion

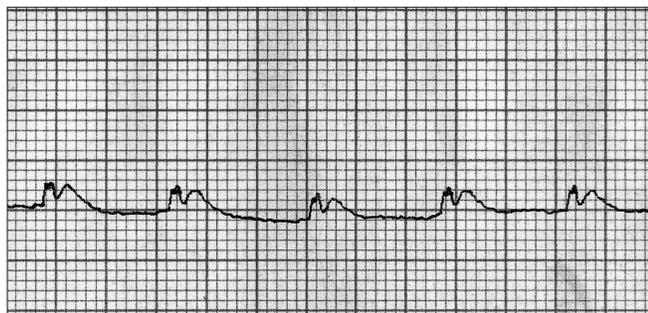
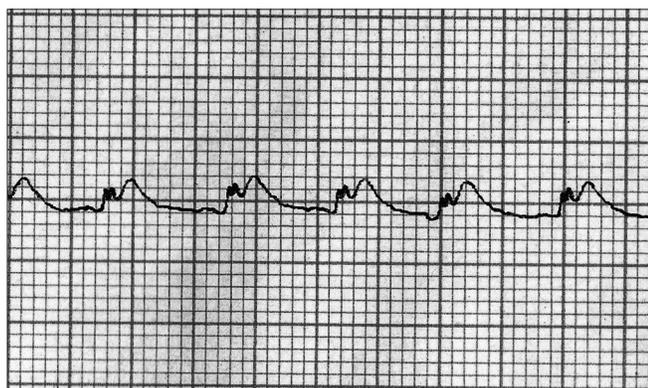
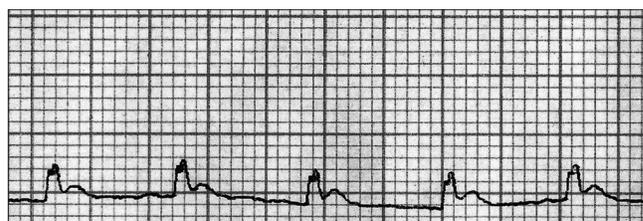
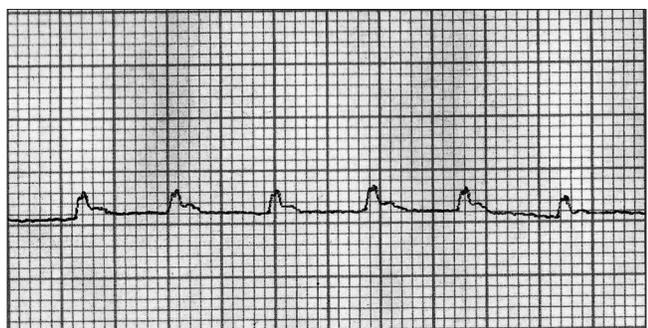
Ghee is the elixir for daily life in human beings. In routine dietetics, ghee is advised either in pure form or medicated or mixed with food articles. However, being animal fat, it

is the most averted substance by the physicians of modern medicine due to its property of increasing serum cholesterol. On the contrary, according to Ayurveda, *Ghrita* is a most favored substance, not only as an important medicine in the management of disease but also as an important food ingredient

Table 5: Effect on electrocardiography of heart

| Parameters | Control | AGLD | AGHD | GGLD | GGHD |
|-----------------------------|--------------|---------------|--------------|-------------|--------------|
| PR interval(s) | 0.05±0.003 | 0.03±0.002** | 0.03±0.002** | 0.04±0.003 | 0.03±0.003 |
| QRS interval(s) | 0.04±0.003 | 0.04±0.004 | 0.03±0.003 | 0.03±0.002 | 0.04±0.002 |
| QT interval(s) | 0.08±0.006 | 0.10±0.008 | 0.08±0.004 | 0.08±0.014 | 0.10±0.002 |
| Heart rate(beats/min) | 345.78±13.57 | 368.56±22.28 | 380.78±20.28 | 345.22±8.89 | 328.11±23.78 |
| RR interval(s) | 0.18±0.007 | 0.17±0.013 | 0.16±0.007 | 0.17±0.004 | 0.19±0.013 |
| QTc (corrected QT interval) | 0.20±0.016 | 0.24±0.018*** | 0.20±0.01 | 0.20±0.032 | 0.23±0.008 |

Data expressed as mean±standard error of mean;AGLD: Low Dose of *Avika Ghrita*; AGHD: High Dose of *Avika Ghrita*; GGLD: Low Dose of *Go Ghrita*; GGHD: High Dose of *Go Ghrita* *P<0.05, *P<0.01, *P<0.001 (compared with control group)

**Figure 1: ECG of AGLD, low dose of *Avika Ghrita*; suggestive of shortened PR interval****Figure 2: AGHD, high dose of *Avika Ghrita*; suggestive of shortened PR interval****Figure 3: GGLD, low dose of *Go Ghrita*****Figure 4: GGHD, high dose of *Go Ghrita*****Figure 5: ECG of control group**

in daily diet. The present study was undertaken to address this controversy by administering it for longer duration at both low and high dose levels to know their effect on various ponderal, biochemical, histopathological, and electro-physiological

parameters. Further, to know why *Acharyas* told that *Avika Ghrita* is inferior and *Ahridya*, whether it is really having any such deleterious effects or not, this formulation was selected for a comparative study with *Go Ghrita*. The study was conducted at two doses, namely, low and high dose levels keeping the fact that the dose is used for both therapeutic purpose (clinical dose) and as diet.

As expected, in the control group normal progressive gain in body weight was observed. However, in all the treated groups the observed weight gain was comparatively less. There can be many reasons for the decrease in the body weight. One of them may be interference with the absorption of essential nutrients from the gastrointestinal tract. The second reason may be the increase in the metabolic rate unlinking it from energy formation mechanism (e.g., uncoupling of oxidative phosphorylation) and the third reason may be stimulation of the satiety center in the hypothalamus.

A moderate to significant increase in the fecal fat content was observed in all the four groups. This is obviously due to higher consumptions of ghee part of which might not have been

absorbed. Since there was no difference between the two types of ghee it has no bearing on the outcome of the discussion.

Fats or ghee have an inherent property of increasing the serum triglyceride and cholesterol, but in the present study no such thing happened in spite of using quite high level of ghee consumption for a pretty long period of 45 days. On the contrary significant lowering of serum triglyceride observed in AGLD administered group and insignificantly in all other groups may be indicative of interference with the triglyceride formation and utilization in the body. The decrease may be due to interference with the fat absorption in the jejuno-duodenal junction or higher incorporation to Very Low Density Lipoprotein (VLDL) or higher utilization by the tissue.

In comparison with the ECG of control group [Figure 5], PR interval was found to be shortened in *Avika Ghrita* administered groups [Figure 1, 2]. This interval represents the time the impulse takes to reach the ventricles from the sinus node. Sympathetic activity increase shall lead to decrease and parasympathetic activity increase leads to prolongation of this interval.^[20] The PR interval decrease may be indicative of faster transmission of impulse from the atria to the ventricles. It is not known whether any sympathetic stimulation is involved or not.

In humans and large animals, QT interval varies strongly and inversely with heart rate. Consequently, in clinical practice and human pharmacologic studies it is typical to correct the measured QT interval for heart rate to obtain measures of QT interval, which are heart rate independent (QTc).^[21] A significant prolongation of QTc was observed in AGLD administered group whereas only insignificant prolongation was observed in GGHD administered group [Figures 1, 4]. This shows that both the doses of ghee have cardiac activity modulating potential; however, how exactly it is produced and its implication for clinical conditions needs to be carefully verified.

The mention of *Ahritya* for *Avika Ghrita* in classics may be based on the observation of interference with the cardiac function at least in some patients, however, *Go Ghrita* at higher dose level also has some potential and needs further careful analysis. Furthermore, there were no marked differences between *Avika* and *Go Ghrita*. The one remarkable difference could be the observation of QTc prolongation. This may be the basis for the classics to categorize it as *Ahritya*.

Conclusion

Both *Avika* and *Go Ghrita* have the potential to produce both desirable and undesirable effects. Among the desirable effects is non-observation of hyperlipidemia in spite of giving good amount of fat, which is supposed to be of saturated type. The adverse effects are cardiac changes as per the ECG observations.

Hence it can be suggested that a person with cardiac and renal problems should better avoid both the ghee.

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हिन्दी सारांश

गो घृत तथा आविक घृत के दीर्घकालीन प्रयोग का आल्बिनो चूहों पर तुलनात्मक अध्ययन

दिपाली जे. शुक्ला, हितेश ए. व्यास, महेश कुमार व्यास, अशोक बी. के., बी. रविशंकर

घृत शताब्दियों से भारतीय आहार का प्रधान अङ्ग रहा है। आयुर्वेदीय ग्रन्थों में आठ विविध प्राणियों के दुग्ध से निर्मित आठ प्रकार के घृतों का वर्णन प्राप्त होता है, जिन में से गाय के दुग्ध से बने घी को श्रेष्ठ तथा आविक (भेड़) दुग्ध से निर्मित घृत को निकृष्ट एवं अहृद्य दर्शाया गया है। प्रस्तुत शोधकार्य प्रायोगिक प्राणियों पर गो घृत तथा आविक घृत के चिरकालीन प्रभावों के अध्ययनार्थ संपन्न किया गया। अध्ययन विस्तार आल्बिनो प्रजाति के चूहों पर दो विभिन्न मात्रा के साथ किया गया। औषध प्रयोग लगातार ४५ दिनों तक किया गया। इन प्राणियों का सामान्य व्यवहार, देहभार, महत्वपूर्ण अङ्गों के भार, मल की स्नेहाक्तता, विभिन्न अङ्गों की कोशिकाओं के प्रयोगशालीय परीक्षण, रक्त परीक्षण, इलेक्ट्रोकार्डियोग्राफी इत्यादि मानकों का अभ्यास किया गया। दोनों में से किसी भी द्रव्य का प्राणियों के सामान्य व्यवहार, देहभार, विभिन्न अङ्गों के भार तथा कोशीय रचना पर विशेष प्रभाव प्राप्त नहीं हुआ, किन्तु आविक घृत की दोनो मात्राओं से प्राणियों में ट्राइग्लिसराइड विशेष रूप से कम होता हुआ पाया गया तथा इलेक्ट्रोकार्डियोग्राफी में भी परिवर्तन पाये गये। संभव है कि आचार्यों द्वारा आविक घृत को अहृद्य दर्शाने का यही कारण हो।

Announcement

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